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# PERSISTENCE AND EFFECTIVE HALF-LIFE OF CHEMICAL WARFARE AGENT VX ON GRASS FOLIAGE

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The primary focus of this investigation was to experimentally determine the Effective Half-Life of VX on grass foliage (*Echinochloa crus-galli*) using living healthy plants to obtain results applicable to VX-contaminated battlefields. The Effective Half-Life of VX on grass foliage was determined as the net effect of factors affecting the persistence of stabilized Chemical Agent Standard Analytical Reference Material (CASARM)-grade VX on grass, including fixation, transformation, and evaporation. Fate of VX on grass foliage was established as the partitioned percentages of the disseminated-VX. Rapid reactions with foliage within the first minute caused 9% of disseminated-VX to quickly become unextractable; however, 88% remained in the form of extractable-VX 1 min post-dissemination. The Effective Half-Life of that extractable-VX on the grass foliage, from 1 min post-dissemination onward, was 72 h (95% confidence interval, 46–98 h). The Effective Half-Life of VX is a key strategic value because it accounts for the persistent VX-hazard from the bulk of disseminated-VX, corresponding to a slow decline over time. Experimental data reported herein were established under controlled and measured environmental conditions, for input into predictive models, comparison to model outcomes, and to provide critical information for decision-making affecting Soldiers on VX-contaminated battlefields.

**15. SUBJECT TERMS**
Chemical warfare agent (CWA)
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## PREFACE

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# PERSISTENCE AND EFFECTIVE HALF-LIFE OF CHEMICAL WARFARE AGENT VX ON GRASS FOLIAGE

## 1. INTRODUCTION

Developing defensive capabilities against chemical warfare agents (CWAs) disseminated into natural environments requires recognition of underlying principles involving CWA interactions with the environment. Commanders who have Soldiers under battlefield conditions must be armed with functional information for "Go/No-Go" decisions related to the exposure of Soldiers to CWAs on agent-contaminated battlefields. However, few experimental data exist that describe interactions between CWAs and terrestrial plants. Archival CWA field study investigations have provided some useful information involving agent–plant interactions. However outdoor testing has inherently uncontrolled environmental test conditions, and by international agreements is generally no longer permitted in the United States. Furthermore, variance in environmental parameters can make the data resulting from outdoor tests less reliable or not useful as input for models that predict outcomes using established scientific and empirical relationships.

Methods for investigating CWA–plant interactions have been successfully developed using living plant species for extended periods of time (Checkai et al., 2017), necessary in order to maintain plant physiological responses and obtain results applicable to CWA-contaminated battlefields. Controlled experimental conditions permit investigations to concentrate more directly on CWA–plant interactions. The research presented in this report focuses on the determination of the Effective Half-Life of *O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate (VX) on contaminated living plant foliage. Physiologically healthy living plants were required in order to investigate and record critical parameters for the effects of CWA–plant interactions (Simini et al., 2016; Haley et al., 2016, 2017), including determination of the Effective Half-Life on plant foliage, a measure of the net effect of factors affecting CWA persistence, including fixation (unextractable bound-CWA non-regenerative to original form), transformation (CWA degradation other than fixation), and evaporation.

The fate of chemical compounds in the environment is influenced by their physical and chemical properties, the environmental material with which the compounds interact, and ambient meteorological conditions (Talmage et al., 2007). Persistence on plant foliage is also a function of the biochemical, physiological, and micromorphological properties of the plant leaves, including leaf epicuticular waxes and cuticle (Sanyal et al., 2006; Simini et al., 2016). The extent of persistence, penetration (absorption), or evaporation of chemical droplets on foliage will depend on vapor pressure, the hydrophilic nature of the compound, and the extent of hydrophobicity of the foliar surfaces (Deseret Test Center, 1970; Willis and McDowell, 1987). Thus, the extent of absorption and persistence of CWAs on foliar surfaces and within plant tissues will vary among different plant species (Gorzkowska-Sobas, 2013), and depend on the CWA disseminated (Deseret Test Center, 1970), just as these factors commonly affect pesticide persistence on foliage (Willis and McDowell, 1987).

The primary focus of this investigation was the determination of the Effective Half-Life of VX on contaminated grass foliage (*Echinochloa crus-galli* [L.] P. Beauv;[1,2] commonly referred to by many names, including barnyard grass, Japanese millet, and wild millet). Other critical parameters for assessing threat from VX on battlefields in natural environments include the visual characterization of the effects of VX on grass foliage (Simini et al., 2016), coefficient of VX wash-off from rainfall (Haley et al., 2016), and contact transfer (exposure) of VX from contaminated foliar surfaces onto Army Combat Uniform (Haley et al., 2017). Results of these additional investigations are detailed in the corresponding technical reports, and together with this report provide critical parameter input for predictive models, direct experimental determinations for comparison of model outcomes, and information for decision-making affecting Soldiers on VX-contaminated battlefields.

## 2. METHODS

### 2.1 Chemicals

The CWA disseminated in this investigation of the Effective Half-Life on grass foliage was VX at 90.3% purity, Chemical Agent Standard Analytical Reference Material (CASARM) grade, Chemical Abstracts Service (CAS) no. 50782-69-9 (U.S. Army Edgewood Chemical Biological Center [ECBC]; Aberdeen Proving Ground, MD), stabilized with 5% by weight diisopropylcarbodiimide (CAS no. 693-13-0; Sigma-Aldrich Company; St. Louis, MO). VX analytical calibration standards and the fluoro derivative *O*-ethyl methylphosphofluoridate (VX-G), along with deuterium-labeled VX (VX-$d_5$) and the deuterium-labeled fluoro derivative $^2H_s$-*O*-ethyl methylphosphofluoridate (VX-G-$d_5$) were obtained from ECBC. The corresponding VX purity determination and verification were by nuclear magnetic resonance spectroscopy (NMR). Reagent-grade isopropyl alcohol (IPA; CAS no. 67-63-0; Sigma-Aldrich Company) was used as the primary extractant of VX from grass. Water used in experiments was ASTM Type I (18 MΩ cm) (ASTM, 2004) that was subsequently allowed to naturally equilibrate with air at ambient conditions.

Miracle-Gro Water Soluble All Purpose plant food (Scotts Miracle-Gro Company; Marysville, OH) fertilizer (24% total nitrogen [calculated as N], 8% available phosphate [calculated as $P_2O_5$], 16% soluble potash [calculated as $K_2O$], 0.02% boron, 0.07% copper [water soluble], 0.15% iron [chelated], 0.05% manganese [chelated], 0.0005% molybdenum, 0.06% zinc [water soluble], and 1.14% ethylenediaminetetraacetic acid chelating agent) was used to prepare dilute phytonutrient solution (530 mg/L) with ASTM Type I water.

All other solvents and reagents were analytical grade or purer and were obtained from Sigma-Aldrich Company.

[1] L. indicates that Carl Linnaeus is the authority for the species name.
[2] P. Beauv. indicates that Palisot de Beauvois was the author of this botanical name.

## 2.2 Plant Selection and Culture

We selected the grass species *E. crus-galli* (Figure 1) for the agent–plant interaction research described in this report and in corresponding investigations (Simini et al., 2016; Haley et al., 2016, 2017). Grass is the most prevalent type of higher plant worldwide, and *E. crus-galli* is one of the most ubiquitous grasses globally (CABI, 2017). *E. crus-galli* is tolerant of both dry and wet natural habitats and is used as forage for grazing animals, as well as for wildlife food and habitat (USDA–NRCS, 2015). This species is also one of the most-important weed species in crop systems globally, and in some parts of the world, *E. crus-galli* is cultivated as a crop for human consumption (CABI, 2016). We used novel methods that we developed to enable and sustain the culture of living, physiologically healthy plants within a chemical agent surety hood (Checkai et al., 2017).

Figure 1. *E. crus-galli* grass plants in native habitat.

Within an environment-controlled plant-growth chamber (model PGC-9/2; Percival Scientific; Perry, IA), *E. crus-galli* grass seeds (lot no. PM11452Q, 2014; Prairie View Nursery; Winona, MN) were germinated by hydration with ASTM Type I water, in potting mix (Miracle-Gro Moisture Control potting mix; Scotts Miracle-Gro Company), within flower pots. The bottoms of the 100 mm (4 in.) diameter pots had been lined with two pieces of absorbent paper, then filled with 170 g (77.1 g dry mass) of the potting mix.

After 7d post-germination individual grass plant seedlings were each transplanted into a pot containing potting mix and grown to mature leaf stage within an environment-controlled plant-growth chamber (Simini et al., 2016). Dilute phytonutrient solution was administered to the plants every 2 to 3 days in order to avoid effects of nutrient deficiencies that may exist in soil, maintain the respective initial moisture mass of each replicate plant system,

and sustain healthy plants. When the individual grass plants had developed two to three fully-mature leaves (Figure 2), the individual seedlings in pots were transferred into the plant culture conditions maintained within a surety hood (Checkai et al., 2017). Both the selection of individual plant and the pot position within the surety hood were randomized for the experimental determination of the Effective Half-Life of VX on grass foliage. A sampling that consisted of six individual replicate leaves from *E. crus-galli* mature grass foliage was taken from additional plants that did not receive VX, to determine the moisture status (water content) of the grass by mass (weight).

Figure 2. *E. crus-galli* grass plants with two to three fully mature leaves, shown within the environment-controlled plant-growth chamber prior to transfer into surety hood.

## 2.3 Dissemination of VX Droplets onto Leaves and Foliage Sampling Times

Plant stands were constructed to hold the pots and plants in fixed positions within the surety hood. A hole was cut in each Petri dish cover, and a pot was placed through the hole and onto the Petri dish (Figure 3). Each pot was secured to a ring stand with an adjustable clamp. Grass leaves near the top of the plant canopy were laid horizontally across a ring, and each of the selected leaves was secured to a ring by a length of clear cellulose acetate tape that was folded in half lengthwise (thereby preventing sticky contact of the acrylate adhesive with leaf surface) and placed across the leaf surface. Ends of the folded tape were then secured to the ring with additional tape to maintain slight pressure against the leaf surface (Figure 4).

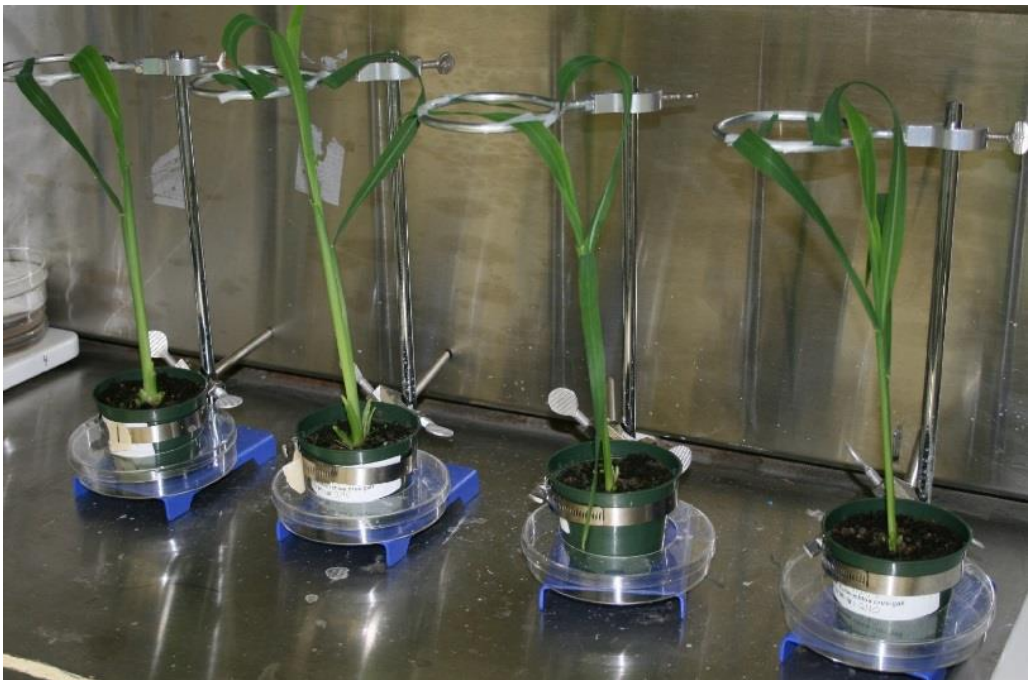

Figure 3. *E. crus-galli* grass plants in surety hood with plant stands.

Figure 4. Close-up view of *E. crus-galli* leaves secured in horizontal position using tape folded in half lengthwise, thereby preventing leaf contact with adhesive. VX was disseminated onto the portion of the grass leaf held within the ring.

This method of securing individual leaves in a horizontal position ensured that disseminated agent droplets contacted the leaf surface at the point intended, and that those locations were easily identified for further investigation. This method also prevented any possible leaf surface damage caused by tape contact or removal. Individual leaves on the living plants remained secured in this horizontal position during and after dissemination of VX. Physiologically healthy plants were maintained within the chemical agent surety hood environment (Checkai et al., 2017). Temperature within the surety hood was maintained at 22 ± 2 °C, and the relative humidity was maintained at 50 ± 10%. Average hood airflow was 1.50 ± 0.09 mph (2.41 ± 0.14 km/h), measured at the face of the hood using an AirData multimeter (model ADM-870C; Shortridge Instruments, Inc.; Scottsdale, AZ). Photosynthetically active radiation (McCree, 1972, 1981) was provided using an adjustable lighting system containing modern, high-intensity light-emitting diodes (Checkai et al., 2017).

Single, nominal 1 µL droplets (approx. 1.2 µL) of stabilized CASARM-grade VX (1.1915 ± 0.0356 mg of VX; average ± standard deviation [SD]), which is a droplet size expected from CWA dissemination under field conditions (TOP, 2011), were individually dispensed using a calibrated 10 µL Hamilton (Reno, NV) gastight syringe. Droplets were disseminated onto grass foliage, and additional droplets were deposited into solvent for analytical quantitation as positive controls of VX dissemination. A single droplet of VX was applied onto a single living leaf that was still attached to a healthy living plant (Figure 4). These methods of leaf stabilization and VX dissemination prevented droplets from merging on the foliage (for the purpose of subsequent analytical determinations). The disseminated-VX droplets were allowed to equilibrate on the grass foliage for 0.017 (1 min), 1, 24, 48, 120, and 168 h before replicate VX-contaminated leaves and respective negative (no VX) control leaves were removed from plants at specified times for analytical determination of VX. On the basis of preliminary experiments, these sampling times were selected to bracket the Effective Half-Life value for VX on grass foliage. Each experimental unit for each sampling time was replicated in quadruplicate ($N$ = 4), with quadruplicate replicates ($N$ = 4) of positive controls of VX dissemination (1, 24, 48, 120, and 168 h). Corresponding analytical determinations were each measured three times ($N$ = 3). The average of the respective quantitations was reported for each experimental replicate, and analytical results for all standards and samples were reported as a mass (mg) of target analyte. Statistical analyses were performed on the resulting experimental data to determine the Effective Half-Life of VX on grass foliage.

## 2.4 Sampling of Contaminated Grass Leaves, Preparation, and Quantitation of VX

Both VX-contaminated and untreated control leaves were removed from grass plants 14 cm (5.5 in.) from the leaf apex (tip), the portion of the leaf that included the location of the VX droplet dissemination, at 0.017, 1, 24, 48, 120, and 168 h post-dissemination, for preparation for analysis. Individual leaves were placed into separate Kapton sample bags (American Durafilm; Holliston, MA). Those sample bags were then sealed with screw-caps, and immediately immersed in liquid nitrogen. The sample bags were removed from the liquid nitrogen, and each screw-cap was replaced with a glass tissueTube culture tube (Covaris; Woburn, MA). The sample bags, with tubes attached, were then placed back into liquid nitrogen. Each leaf was cryogenically pulverized within the Kapton bag in a cryoPREP impactor

(Covaris), then directly transferred into the attached glass culture tube of the tissueTube device and weighed within the tube of the tared device. Then 10 mL of IPA was quantitatively added into the tube containing the pulverized leaf tissue, and VX was extracted using a Covaris S2 Focused acoustic ultrasonicator. Table 1 lists the ultrasonic parameters used in processing leaf tissue by extraction.

Table 1. Covaris S2 Focused Acoustic Ultrasonicator Settings Used to Extract Grass Leaves

| Stage | Duty Cycle (%) | Intensity Setting | Cycles/Burst | Duration (s) |
|---|---|---|---|---|
| 1 | 10 | 10 | 1000 | 20 |
| 2 | 10 | 10 | 1000 | 20 |
| 3 | 10 | 10 | 500 | 20 |
| 4 | 10 | 10 | 500 | 20 |

After ultrasonic extraction, the tube was centrifuged at room temperature for 15 min (relative centrifugal force [RCF] of 3645 ×*g*). The resulting extract was separated from the pellet of leaf tissue and mixed. A sub-sample of the extract was quantitatively removed, quantitatively spiked withVX-$d_5$ internal standard, and diluted with IPA as necessary.

The pellet of each leaf tissue was then independently washed twice with 10 mL aliquots of fresh IPA. The ultrasonication and centrifugation portions of the extraction procedure were repeated for each of these two pellet-washes, along with the subsequent addition of VX-$d_5$ internal standard into the extract for analysis of each pellet-wash, with dilution by addition of IPA as necessary. Furthermore, following the two pellet-wash extractions, the Kapton sample bag was then rinsed with 1 mL of IPA. That rinsate was transferred into a polypropylene microcentrifuge tube, centrifuged (RCF of 9279 ×*g*), and spiked with VX-$d_5$ internal standard, for independent analysis.

Quantitative analytical determinations of the VX extracted from the grass foliage were conducted using high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) by means of instrumentation that consisted of an Agilent 1260 liquid chromatograph and triple-quadrupole mass spectrometer with MassHunter data acquisition and analysis software (Agilent Technologies; Wilmington, DE). The HPLC was fitted with an Agilent Eclipse XDB-$C_{18}$ column (5 µm, 4.6 × 150 mm). Sample injections were 1 µL. A 13 min separation method was used; the composition of mobile phase A was 0.1% (v/v) formic acid in $H_2O$ and mobile phase B was 0.1% (v/v) formic acid in methanol. The gradient conditions used for HPLC separation are presented in Table 2.

The HPLC column eluent was delivered to an electrospray ionization source maintained in positive ion mode. MS/MS discrimination was performed via the multiple reaction monitoring (MRM) technique that incorporated isotope dilution (VX-$d_5$) and used the following three mass transitions: VX-$d_5$ internal standard, VX quantitation, and VX confirmation (Table 3).

Table 2. HPLC A–B Mobile Phase Gradient for VX Quantitation

| Time (min) | Mobile Phase Solvent Ratio (as %B) |
|---|---|
| 0 | 0.1 |
| 2 | 0.1 |
| 7 | 95 |
| 8 | 95 |
| 11 | 0.1 |
| 13 | 0.1 |

Table 3. MRM Mass Transitions for VX Quantitation

| Analyte Determined | Mass (Da) | |
|---|---|---|
| | Precursor | Product |
| VX-$d_5$ internal standard | 273 | 128 |
| VX quantitation | 268 | 128 |
| VX confirmation | 268 | 86 |

Calibration for quantitation was conducted by plotting the relative responses of VX and VX-$d_5$ as a function of concentration. An 11 point calibration curve (5.0–5000 ng/mL VX, $r^2$ = 0.9992+) was constructed, and a linear fit was applied (1/$x$ weighting) (McGuire et al., 2016; Byers et al., 2008). Reported VX concentrations were calculated by applying the equation of fit and dilution factors, as applicable.

Quantitation of releasable bound-VX residual in each grass leaf tissue pellet, after the pellet had been twice-washed by subsequent ultrasonication re-extraction with IPA (described above), was accomplished using a fluoride regeneration method. The fluoride regeneration procedure involved adding 1 mL of 10 mM acetate buffer (pH 4) and 200 µL of 6 M KF into each glass tube of the tissueTUBE device containing a washed grass leaf tissue pellet that had undergone pellet-wash extraction. The sample was then extracted with the fluoride ion solution mixture using a Covaris S2 Focused acoustic ultrasonicator and centrifuged (as described above). The fluoride ion regeneration extract (regen/extract) was separated from the leaf pellet, and the regen/extract solution was transferred into a 15 mL polypropylene centrifuge tube. Addition of the regeneration reagents, ultrasonic extraction, centrifugation, and transfer of resulting regen/extract solution were repeated, and these two regen/extracts were combined for analytical determination of bound-VX residual that had been freed by fluoride ion to form the organophosphonofluoridate moiety of VX, *O*-ethyl methylphosphonofluoridate (VX-G). Formation of VX-G in the presence of such an excess of fluoride ion is quantitatively a one-to-one reaction with bound-VX that is residual within tissue. This fluoride regeneration method for bound-VX residual within plant leaf tissue is an adaptation of the method originally developed to detect the presence of VX in biological samples from mammals that had experienced an exposure to VX vapor (McGuire et al., 2016; Byers et al., 2008; Degenhardt et al., 2004) and is a highly sensitive method for detecting VX in biological matrices (McGuire et al., 2016; Byers et al., 2008).

An aliquot of the regen/extract was then removed quantitatively, spiked with VX-G-$d_5$ internal standard, and subjected to solid-phase extraction (SPE) using an Oasis HLB 3 cc (60 mg) flangeless SPE cartridge (Waters Corporation; Milford, MA) that was conditioned with 1 mL of ethyl acetate (EtOAc), then 1 mL of IPA, and finally 1 mL of 10 mM acetate buffer (pH 4). The VX-G and VX-G-$d_5$ internal standard were eluted from the cartridge with EtOAc, then the eluent was dried over anhydrous sodium sulfate. The regen/extract eluent was concentrated to approximately 50 µL.

Quantitative analysis of fluoride ion regenerated-VX (VX-G) from grass leaf tissue was performed using gas chromatography with tandem mass spectrometry (GC/MS/MS) by means of instrumentation that consisted of an Agilent 7000A GC/MS/MS triple-quadrupole mass spectrometer with MassHunter data acquisition and analysis software. Gas chromatographic separations were achieved using an Rtx-1701 column (30 m × 0.25 mm × 25 µm) (Restek Corporation; Bellefonte, PA). The carrier gas was helium, and the flow rate was 1 mL/min. Splitless injections of 3.0 µL were performed using an Agilent 7693 automatic liquid sampler autoinjector at a temperature of 225 °C. The oven temperature program is presented in Table 4.

Table 4. GC Oven Temperature Program for VX-G Quantitation

| Stage | Temperature (°C) | Ramp (°C/min) | Hold Time (min) |
|---|---|---|---|
| 1 | 35 | – | 0.1 |
| 2 | 100 | 15 | 0 |
| 3 | 275 | 35 | 3 |

Injected samples were ionized by positive-ion chemical ionization with ammonia reagent gas. The detector was operated in MRM mode, and transition responses were obtained at a dwell time of 200 ms for each transition (Table 5). Helium was used as the collision gas, and the collision energy was 10 eV.

Calibration was conducted by plotting the relative responses of VX-G and VX-G-$d_5$ as a function of concentration. An 11 point calibration curve (0.5–1000 ng/mL of VX-G; $r^2$ = 0.9975+) was constructed, and a linear fit was applied (1/$x$ weighting) (McGuire et al., 2016; Byers et al., 2008). Reported VX-G concentrations were calculated by application of the equation of fit and dilution factors, as applicable.

Table 5. MRM Mass Transitions for VX-G Quantitation

| Analyte | Mass (Da) | |
|---|---|---|
| | Precursor | Product |
| VX-G-$d_5$ internal standard | 149 | 100 |
| VX-G quantitation | 144 | 99 |

Untreated sections of VX-treated leaves (i.e., the other half of a treated leaf), between the culm and the 14 cm apical leaf section that received a VX droplet, were also collected, frozen in liquid nitrogen, processed by primary extraction with IPA using focused ultrasonication, and analyzed for VX via HPLC/MS/MS. These samples were collected at 48 and 168 h post-dissemination of VX onto grass leaves.

## 2.5 Quantitation of VX Hydrolysis Products Ethyl Methyl Phosphonic Acid (EMPA) and Methylphosphonic Acid (MPA)

VX reacts with moisture in the environment to form the primary hydrolysis product, EMPA (Talmage et al., 2007). EMPA is considered relatively stable in the environment (Timperly et al., 2001), and it reacts exceptionally slowly with water to form a secondary hydrolysis product, MPA.

Concentrations of EMPA in individual grass leaves were analytically determined using a 25 µL sub-sample of each initial IPA-extraction of VX from each ultrasonicated grass leaf tissue extract, by injecting the extract directly onto an ion chromatograph for separation and quantitation of EMPA. An IonPac AS18 column (4 × 250 mm) with suppressed conductivity detection was used (Dionex Corporation; Sunnyvale, CA). Separation of EMPA from other anionic species (e.g., sulfate, chloride, fluoride) in the IPA extract of grass foliage was accomplished using a KOH gradient elution at a flow rate of 1 mL/min (Table 6). A linear calibration curve for EMPA was generated by injecting external standards of EMPA at concentrations of 1.23, 1.47, 2.45, 4.90, 9.80, and 14.7 µg/mL ($r^2$ = 0.9994+). These extracts were also screened for the presence of MPA, by comparable analysis.

Table 6. Gradient Conditions for Ion Chromatographic Quantitation of EMPA in Grass Leaves

| Time (min) | KOH (mM) | Curve Fit |
|---|---|---|
| 0–2 | 5–10 | Linear |
| 2–8 | 10–30 | Linear |
| 8–10 | 30–45 | Linear |
| 10–12 | 45–5 | Linear |

## 2.6 Statistical Analyses of Data

Data for VX extracted from grass leaves as a function of time post-dissemination were statistically analyzed using regression models selected from among those described in the Environment Canada Guidance Document EPS 1/RM/46 (2005). During the mathematical model selection process, compliance with the normality assumptions and homoscedasticity of the residuals were determined by examining the stem-and-leaf graphs and histograms of the residuals. The best fit was evident when the regression lines generated by the models were closest to the data points, the regression coefficients for point estimates were the greatest, the residuals were homoscedastic (i.e., had most random scattering), and the means, standard errors, and variances of the residuals were the smallest. The 95% confidence intervals (CIs) associated with the point estimates were determined.

Analysis of variance (ANOVA) was used on the data for the values of extracted-VX. Mean separations were determined using Fisher's least-significant difference (LSD) pairwise comparison test. All statistical analyses were done using untransformed data and analytically determined values of the extracted mass of disseminated-VX that was recovered. Data were screened to identify unusual values that could be distorting results and to identify outliers, typically with a Studentized residual $\geq \pm 2$. None of the data from the present study were excluded. A significance level of $p \leq 0.05$ (95% confidence level) was accepted for all statistical tests. Statistical analyses were performed using SYSTAT 11 software (Systat Software; Chicago, IL).

## 3. RESULTS

### 3.1 Persistence of VX on Grass Foliage as a Function of Time

The percent moisture content of the fully mature *E. crus-galli* grass leaves prior to VX dissemination was determined to be 88% water (88.1 ± 1.1%), based on a sampling of mature grass foliage from plants that did not receive VX.

Upon dissemination, a VX droplet immediately sorbed into the live mature grass leaves upon contact with the leaf surface, producing an appearance of wetness within the affected area. Over the course of a minute ($t$ = 1 min), this wet appearance spread outward in all directions from the initial location of the absorbed droplet but favored migration parallel to the leaf veins (Simini et al., 2016). After 1 min, the area of the leaf central to droplet dissemination appeared noticeably discolored (Figure 5). Spreading of the VX droplet within the leaf continued for up to 1 h, and by that time the VX-affected area of the leaf surface appeared dark-colored and dry, apparently due to initiation of cell lysis (Figure 6). After 24 h, cell necrosis was evident within the affected area (Figure 7).

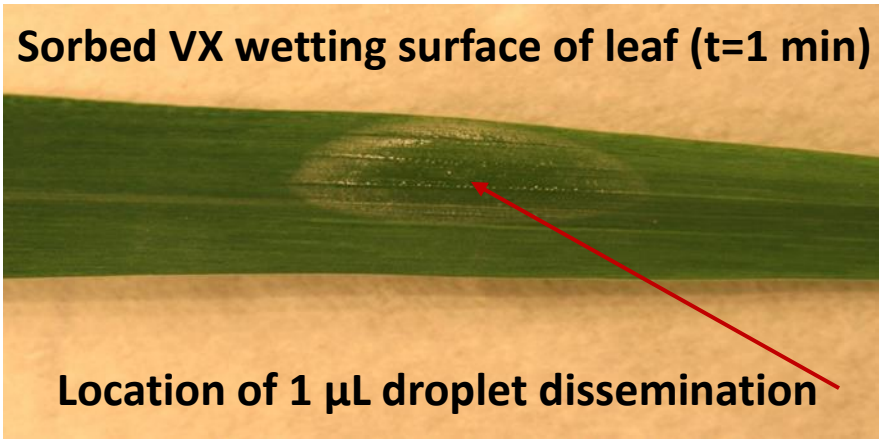


Figure 5. Visual appearance of VX on leaf of a living grass plant (*E. crus-galli*) 1 min post-dissemination of a 1 µL droplet of stabilized CASARM-grade VX onto the leaf.

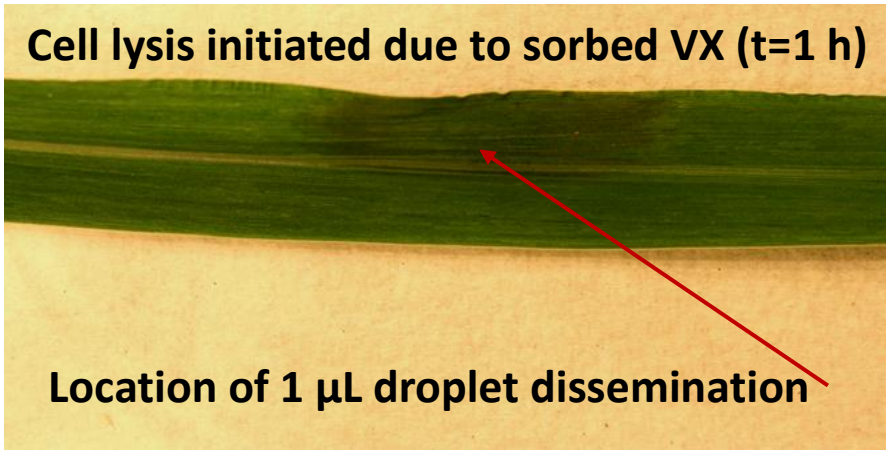


Figure 6. Visual appearance of VX on leaf of a living grass plant (*E. crus-galli*) 1 h post-dissemination of a 1 µL droplet of stabilized CASARM-grade VX onto the leaf.

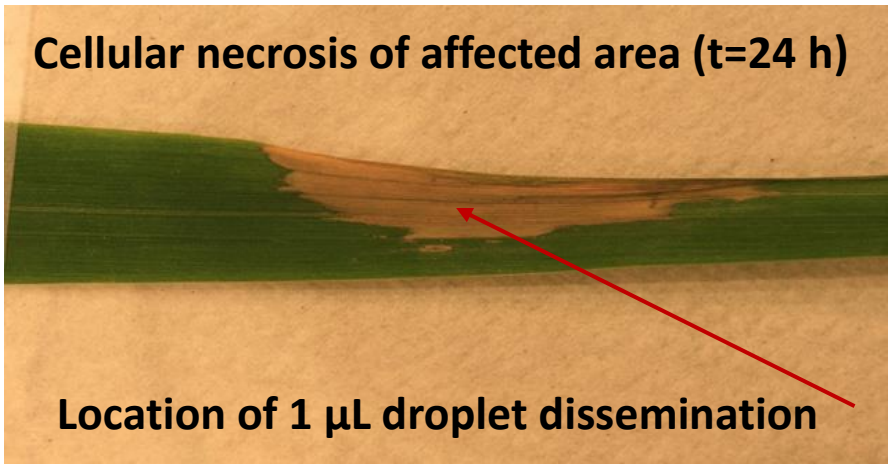


Figure 7. Visual appearance of VX on leaf of a living grass plant (*E. crus-galli*) 24 h post-dissemination of a 1 µL droplet of stabilized CASARM-grade VX onto the leaf.

VX-contaminated leaves (1 µL droplet) and negative-control (untreated) leaves were individually removed from grass plants at 0.017, 1, 24, 48, 120, and 168 h post-dissemination for preparation and analysis. The positive controls of VX dissemination were also prepared by quantitative addition of internal standard into individual 1 µL droplets of stabilized CASARM-grade VX in solvent, to analytically determine the mass of VX disseminated, yielding 100 ± 3% disseminated. The results of these analyses and the corresponding mass balance values (the net percent recovery of all forms of VX, plus EMPA) are shown in Table 7. MPA was not detected in any of the analyzed grass leaf samples (<0.2% of disseminated-VX) over the course of 168 h post-dissemination. At 1 min post-dissemination, approximately 88% (88.1 ± 3.0%) of the disseminated-VX within the contaminated foliage remained in the form of extractable-VX, and approximately 3% (2.9 ± 1.7%) remained as bound-VX in a regenerative (regen/extract) form. Approximately 91% of the total disseminated-VX was accounted for by mass balance 1 min post-dissemination. As time post-dissemination proceeded, the percentage of stabilized

CASARM-grade VX that was recovered as VX declined (eq 1; Figure 8), and the proportion of VX that hydrolyzed to EMPA slowly increased. Most of the sampling time points yielded significantly different ($p > 0.05$) percentages of VX remaining within the grass foliage post-dissemination (Table 7). However, the percentage in the form of extractable-VX within the grass foliage was not significantly different after 1 h compared to that extractable 1 min post-dissemination.

Untreated sections of VX-treated leaves (i.e., the other half of a treated leaf, between the culm and the 14 cm apical leaf section that received a VX droplet) contained trace amounts of VX. These trace amounts were extremely minor compared to the VX in the treated apical leaf section. At 48 h post-dissemination, untreated sections of VX-treated leaves contained two-hundredths of a percent (0.020 ± 0.003%) of the VX that had been disseminated onto the apical leaf sections, and by 168 h post-dissemination, untreated sections contained only five-thousandths of a percent (0.005 ± 0.002%) of the disseminated-VX.

Table 7. Stabilized CASARM-Grade VX on Foliage of Living Grass Plants (*E. crus-galli*) as a Function of Time Post-Dissemination: VX Fate Expressed as Percent of Disseminated-VX ± SD

| Time Post-Dissemination of 1 µL VX Droplet (h) | VX Recovery by Extraction: Grass Foliage (% ± SD) | | | Total VX in Grass Foliage Post-Dissemination[†] (% ± SD); 100 ± 3.0% [a] was disseminated | Regen/Extract $F^-$ Released VX: Residual in Foliage (% ± SD) | EMPA[‡] in Grass Foliage: Percent of Applied VX (% ± SD) | Mass Balance: Total Percent of VX Accounted for in Grass Foliage (%) |
|---|---|---|---|---|---|---|---|
| | Primary Extraction | Pellet Wash Extraction | Kapton Bag Rinse | | | | |
| 0.017 | 65.2 ± 6.1 | 15.0 ± 11.1 | 7.9 ± 7.5 | 88.1 ± 3.0 [b] | 2.9 ± 1.7 | BDL[¥] | 91.0 |
| 1 | 60.7 ± 4.1 | 15.9 ± 3.9 | 2.2 ± 0.8 | 78.8 ± 2.5 [b] | 4.2 ± 2.2 | BDL[¥] | 83.0 |
| 24 | 48.9 ± 5.6 | 7.6 ± 1.5 | 3.6 ± 1.9 | 60.1 ± 5.2 [c] | 1.8 ± 0.7 | 3.4 ± 0.2 | 65.3 |
| 48 | 40.1 ± 7.9 | 8.2 ± 1.8 | 1.8 ± 0.6 | 50.1 ± 8.8 [d] | 2.9 ± 1.2 | 4.1 ± 0.5 | 57.1 |
| 120 | 27.2 ± 11.5 | 3.8 ± 2.5 | 1.2 ± 0.5 | 32.2 ± 13.6 [e] | 2.5 ± 0.9 | 4.5 ± 1.3 | 39.2 |
| 168 | 25.8 ± 3.0 | 5.1 ± 1.5 | 1.4 ± 0.5 | 32.3 ± 2.9 [e] | 2.6 ± 1.2 | 10.3 ± 1.6 | 45.2 |

[†]Values with the same letter are not significantly different ($p > 0.05$) by ANOVA and Fisher's LSD means comparison test.

[‡]VX hydrolysis to EMPA occurs as a 1:1 molar reaction; here, EMPA is expressed as the percent of disseminated-VX extracted as EMPA.

[¥]BDL, below detection limit.

## 3.2 Effective Half-Life and Fate of VX on Grass Foliage

Data for VX extracted from grass leaves as a function of time post-dissemination were statistically analyzed using regression models from among those described in Environment Canada Guidance Document EPS 1/RM/46 (EC, 2005). Two of these models had good fits to the data for extractable-VX (Table 8). A further discussion of model results is provided in the appendix.

Table 8. Parameters of Fit-to-Data for Nonlinear Regression Models Describing VX Extractable from Grass Foliage Post-Dissemination

| Fit Parameter | Logistic Gompertz Model | Exponential Model |
|---|---|---|
| Raw $r^2$ | 0.988 | 0.988 |
| Mean corrected $r^2$ | 0.913 | 0.906 |

The mathematical model that yielded best fit to the extractable-VX data, based on the mean corrected $r^2$ value (0.913), was the logistic Gompertz model:

$$Y = a \times e^{([\log(1-p)] \times [H/ETp]b)} \quad (1)$$

where:

- $Y$ = number for a measurement endpoint (i.e., percent recovery),
- $a$ = Y-axis intercept,
- e = base of the natural logarithm,
- $p$ = desired value for "p" percent recovery (e.g., 0.5 for 50% recovery),
- H = time duration (hours) of the recovery,
- ETp = estimate of time (hours) for a specified percent recovery, and
- $b$ = scale parameter that defines the shape of the equation.

During the first minute post-dissemination approximately 12% (11.9 ± 3.0%) of the disseminated-VX reacted rapidly with plant tissue, initiating disruptive discoloration of the leaf. The resulting VX product of that reaction was either fixation with plant tissue or degradation of VX to form a product other than EMPA (Table 7). One minute post-dissemination, the percentage of VX that had hydrolyzed to EMPA was below the corresponding detection limit. Of the 12% of VX that rapidly reacted with grass foliage upon dissemination, approximately 3% of the disseminated-VX was recovered in the regen/extract at 1 min post-dissemination, with the other 9% apparently in fixation with plant tissue, assuming that any other transformation products would have been recovered in the regen/extract (McGuire et al., 2016; Byers et al., 2008). Thus, the resulting net recovery (mass balance) of the disseminated-VX was 91% at 1 min post-dissemination, with 88% present as VX. As time proceeded post-dissemination, the percentage of stabilized CASARM-grade VX that was recovered as VX declined in a nonlinear manner (eq 1; Figure 8), and VX hydrolyzation to EMPA increased very slowly. After 1 h post-dissemination the proportion of disseminated-VX remaining in the form of VX had not significantly declined ($p > 0.05$) from the amount of VX extractable 1 min post-dissemination (Table 7).

The mass (mg) of VX recovered from contaminated grass leaves, as a function of time post-dissemination, is shown for replicated experimental data points in Figure 8, with sampling points bracketing the time end-point for 50% recovery of VX. The curve shown in Figure 8 is the logistic Gompertz mathematical model which best fit the experimental data.

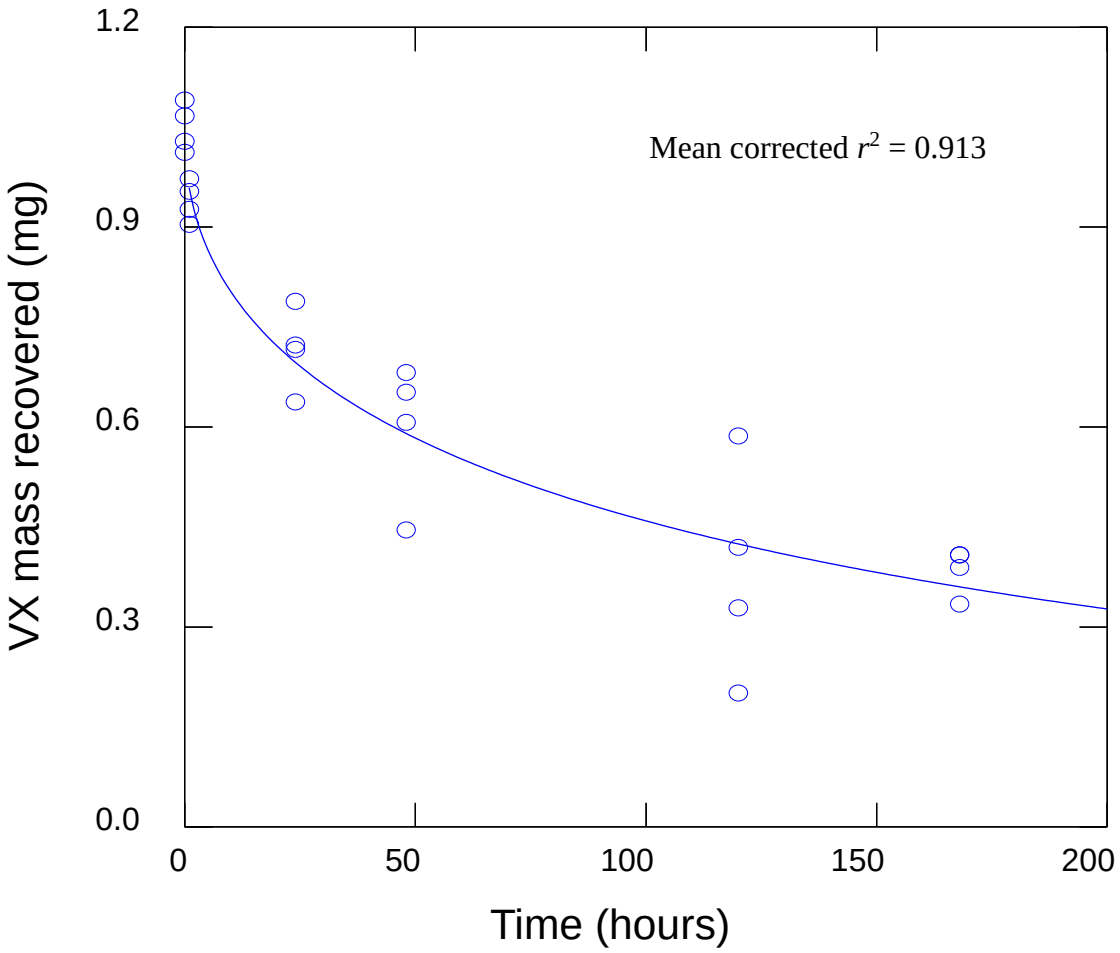


Figure 8. Mass recovered (mg) in the form of VX from contaminated grass foliage as a function of time post-dissemination of 1 µL droplets of stabilized CASARM-grade VX.

The Effective Half-Life of VX for the 88% of VX in grass foliage (*E. crus-galli*), in the form of VX, was determined to be 72 h (95% CI, 46–98 h), on the basis of VX recovered from grass foliage 1 min after dissemination versus the subsequent decline in extractable-VX thereafter. The Effective Half-Life of VX on grass foliage was determined under controlled experimental conditions as the net effect of factors affecting the persistence of the VX, including subsequent fixation, transformation, and evaporation. When the initial rapid reaction of 9% of VX in fixation with plant tissue is included (i.e., in comparison to the total amount of VX disseminated), the time interval for half of the disseminated-VX to remain identifiable in the form of VX was 42 h (95% CI, 25–60 h), similarly utilizing the logistic Gompertz model.

## 4. DISCUSSION

VX is an oily liquid that is relatively nonvolatile and slow to hydrolyze under most ambient environmental conditions; thus, VX may persist for weeks or longer in the environment thereby causing long-term contamination due to slow rates of oxidative or hydrolytic transformation (Groenewold, 2010). Rates of degradation reactions can vary substantially in the environment, so the period of time between dissemination and detoxification of VX by natural means is variable (Groenewold, 2010).

In other studies where VX was topically applied onto harvested desert grasses under laboratory conditions, residual VX was chemically extracted for periods of up to several months, and interactions between plant metabolites and VX were reported to be complex (Deseret Test Center, 1970). Time-dependent variances in the physiology of harvested dead plant tissues can make the data from such test parameters less reliable as input for models that predict outcomes for CWA–plant interactions in the field using established scientific and empirical relationships. Because plant metabolites are known to chemically interact with the family of organophosphate compounds that includes VX, investigation of persistence and the Effective Half-Life of VX on plants requires physiologically healthy living plants in order to elucidate and record critical parameters for the effects of VX–plant interactions.

We applied recently developed methods for investigating CWA–plant interactions using living healthy grass plants for extended periods of time (Checkai et al., 2017), which is necessary in order to maintain plant physiological responses and obtain results applicable to VX-contaminated battlefields. Using controlled measured environmental conditions within a surety hood, we evaluated the amount of VX persisting on the foliage of living grass plants (*E. crus-galli*) as a function of time post-dissemination. The Effective Half-Life of VX on grass foliage was determined as the net effect of factors affecting the persistence of stabilized CASARM-grade VX on grass, including fixation, transformation, and evaporation. The fate of VX on grass foliage was established as the partitioned percentages of the stabilized CASARM-grade VX that remained as extractable-VX, hydrolyzed to EMPA, or became bound-VX residual in grass foliage, and that freed into regen/extract by fluoride ion.

Because VX reacts with moisture (water) in the environment to form the primary hydrolysis product EMPA (Talmage et al., 2007; Groenewold, 2010), moisture sustained within living grass foliage can cause hydrolysis of VX; however, foliage constituents can affect the rate of hydrolysis (Love et al., 2004). The percent moisture within the *E. crus-galli* grass foliage that we cultured in a surety hood contained 88% water by weight, prior to VX dissemination onto the foliage. This moisture content comports with the available moisture commonly found in *E. crus-galli* leaves; however, even foliage of drought-stressed living grass plants have been shown to sustain relative water contents in excess of 60% by weight (Hamin et al., 2016). Results in previous reports show that the persistence of VX in aqueous solution varies, depending on the properties of the solution and concentration (ECBC Safety Data Sheet, 2015; Love et al., 2004; Epstein et al., 1974); the half-life for spontaneous hydrolysis of VX in pure water in the absence of pH buffering has been estimated to be 80 h (Yang et al., 1990). Water, including that within grass tissues, may hydrolyze VX to EMPA, the primary product of hydrolysis under normal ambient environmental conditions.

Our data demonstrate that the interaction of VX with *E. crus-galli* leaves on living grass plants involves several reaction pathways that affect the fate, persistence, and Effective Half-Life of VX on grass foliage. Upon contact with the leaf surface the disseminated-VX droplets immediately sorb into the living mature grass leaves, due to the lipophilic properties of VX (ECBC Safety Data Sheet, 2015; Love et al., 2004) and the hydrophobic properties of the grass leaves (Simini et al., 2016). During the first minute post-dissemination approximately 12% of the disseminated-VX reacted rapidly with plant tissue, so that the resulting VX product of that reaction was either fixation within plant tissue or degradation to form a product other than EMPA that was retrievable into regen/extract (Table 7). Yang et al. (1999) have reported that VX is thought to react via electron acceptance by phosphorus within VX by reaction with anionic nucleophiles. During this initial minute of VX contact with leaf tissue it is highly unlikely that an appreciable amount of VX is lost via evaporation, primarily because the vapor pressure of VX is very low (Tevault et al., 2012; ECBC Safety Data Sheet, 2015) and the duration of exposure to air (flowing 1.50 ± 0.09 mph; 2.41 ± 0.14 km/h) of the VX sorbed into leaf tissue is very brief.

During the first minute post-dissemination approximately 12% of the VX rapidly reacted within plant tissue and initiated the disruptive discoloration of the foliage in the location of the VX droplet (Figures 5–7). One minute post-dissemination, the amount of VX that hydrolyzed to EMPA was below the corresponding detection limit. Of the 12% of disseminated-VX that rapidly reacted with grass foliage, approximately 3% of the VX was recovered in the regen/extract at 1 min post-dissemination with the other 9% in fixation with plant tissue, assuming that any other transformation products would otherwise be recovered in the regen/extract (McGuire et al., 2016; Byers et al., 2008). Thus, the resulting net recovery (mass balance) of the disseminated-VX was 91% at 1 min post-dissemination, with 88% present in the form of VX. As time proceeded, the percentage of the stabilized CASARM-grade VX that was recovered as VX declined in a nonlinear manner (eq 1; Figure 8), and the proportion of VX that hydrolyzed to EMPA slowly increased. The decline in the net recovery of the disseminated-VX as a function of time (Table 7) may be attributed to continuing fixation reactions of VX within affected grass tissue (primarily limited to the area of droplet spread [Simini et al., 2016]), and to a small extent, slow evaporation.

Columbus et al. (2012) found that at 20 °C with a wind speed of 1.5 mph (2.41 km/h), comparable to our temperature and wind conditions, VX evaporated very slowly from the surface of bitumen sheets and asphalt. Bituminous pitch consists chiefly of hydrocarbons, so that over the course of many days (>20 days; 480+ h) accumulative evaporation eventually accounted for approximately 30% of the applied mass of pure VX that they initially applied, and 55% of their applied-VX could not be extracted. They attributed unextractable-VX to be the result of dissolution of VX into the organic bitumen along with corresponding degradation processes, including EMPA formation (detected by NMR) beginning 14 days (336 h) post-dissemination. In comparing the results of our investigation of VX dissemination onto living grass foliage (88% water) with that of disseminating VX onto asphalt (primarily hydrocarbons), although post-dissemination we detected EMPA formation earlier in grass foliage than Columbus et al. did in asphalt, the EMPA in grass only accounted for small percentages of the total disseminated-VX (Table 7). In both situations, fixation reactions with organic compounds in the matrix appeared to dominate the long-term fate of VX in the matrix,

with very slow evaporation over time. After 1 week (168 h) post-dissemination onto grass foliage (i.e., when the foliage was necrotic; Figure 7), our net recovery percentage of disseminated-VX in the form of VX was comparable (approximately 45%) to that of Columbus et al. (2012) for asphalt. However, in the short term, the extent of penetration (sorption) into living grass foliage and persistence of VX in foliage depends on the lipophilic nature of VX and the hydrophobicity of the foliar surfaces (Deseret Test Center, 1970; Willis and McDowell, 1987), and the metabolites within the plant tissue (Deseret Test Center, 1970). Thus, the extent of absorption and persistence of CWAs on foliar surfaces, and within plant tissues, will vary among different plant species (Gorzkowska-Sobas, 2013), and depend on the CWA disseminated (Deseret Test Center, 1970), just as these factors commonly affect pesticide persistence on foliage (Willis and McDowell, 1987).

The persistence of VX, both on and in grass foliage, causes concern regarding the potential for contact transfer of disseminated-VX onto Army Combat Uniform (ACU) and the hazard it presents. Proportions of VX transferred from contaminated grass leaves to ACU at 0.017 (1 min), 0.25, 0.5, 1, and 4 h post-dissemination were approximately 71, 5, 0.8, 0.3, and 0.1%, respectively, of the disseminated stabilized CASARM-grade VX (Haley et al., 2017). The 71% of disseminated-VX that was immediately transferable by contact onto ACU 1 min after dissemination onto grass foliage is a large proportion of the VX that was readily extractable (88%; Table 7) from grass foliage 1 min post-dissemination (ratio of immediately transferable VX to that readily extractable was 71%:88%; 0.8). Furthermore, under field conditions, leaf tissues could become damaged by troop movement over VX-contaminated areas, and this would cause release of additional agent from contaminated leaves. Haley et al. (2017) tested this hypothesis, after completion of direct-contact transfer at 4 h post-dissemination, by applying grinding pressure to each ($N$ = 4) contaminated leaf in a back-and-forth motion. The additional amount of VX transferred onto fresh ACU swatches averaged approximately an additional 16 times the amount of VX that was initially transferred at 4 h post-dissemination (Haley et al., 2017). Because the proportionate amount of disseminated-VX that transferred at 4 h post-dissemination increased by more than an order of magnitude following grass foliage damage, from an initial 0.1% to an additional 1.6% transferred, it appears that the majority of the VX disseminated onto grass leaves quickly resides within the foliage. This result comports with both visual evidence (Figures 5–7; Simini et al., 2016) and analytical results (Table 7) that indicate VX continues to slowly react to fixation within grass leaf tissues over time, primarily limited to the area of droplet spread (Simini et al., 2016).

Untreated sections of VX-treated leaves (between the culm and the 14 cm apical leaf section that received a VX droplet) contained only trace amounts of VX. These trace amounts were extremely minor compared to the VX in the treated apical leaf section; at 48 h post-dissemination, untreated sections of VX-treated leaves contained two-hundredths of a percent (0.02%) of the VX that had been applied to the apical leaf section, and by 168 h post-dissemination, untreated sections contained only five-thousandths of a percent (0.005%) of the applied VX.

VX droplet absorption into a porous matrix, when it causes the chemical agent to become out of direct-contact with air, reduces the rate of evaporation of the low-volatility VX (Columbus et al., 2012; Brevett et al., 2009; Groenewold, 2010). VX droplet dissemination onto living grass foliage, its immediate sorption into tissues, and plant responses to injury, may make

the proportion of VX that is subsequently lost by evaporation in the short term (hours to days) from within grass tissues an extremely small proportion of the disseminated-VX. Formation of amorphous callose (β-1,3-glucan) in response to adverse environmental factors is a protective reaction within living plant tissues that can seal off tissues and retard evaporation from affected areas. Leaf injury initiates formation of amorphous callose, which is synthesized by callose synthases (Piršelová and Matušíková, 2013). Callose formation in response to plant injury is mediated by guanine nucleotide-binding proteins and regulated by active plant hormones such as auxin (e.g., indole-3-acetic acid), which controls cell growth and development of plant organs including leaves, and adenine cytokinins (e.g., kinetin, zeatin, and 6-benzylaminopurine), which control cell division, morphogenesis, and leaf senescence, while the rate of callose deposition is regulated by abscisic acid (Piršelová and Matušíková, 2013; Atwell et al., 1999).

Although it is of interest that 9% of stabilized VX rapidly becomes unavailable due to fixation within grass foliage, the bulk of disseminated-VX remains in the form of VX at 1 min post-dissemination. The Effective Half-Life of VX on grass (*E. crus-galli*) was determined to be 72 h (95% CI, 46–98 h), based on VX (mg) recovered from grass foliage when compared to that remaining 1 min after dissemination (1.0498 mg/leaf). Therefore, the Effective Half-Life of VX on grass foliage, from 1 min post-dissemination onward, was 72 h. The Effective Half-Life of VX is a key strategic value because it accounts for persistent hazard from 88% of the disseminated-VX, corresponding to the slow decline of the bulk of VX that remains in grass foliage after 1 min post-dissemination. The net recovery by mass balance of the disseminated-VX was 91% at 1 min post-dissemination, with 88% present in the form of VX. When compared to the total amount of VX disseminated (1.1915 mg/leaf), the time interval for half of the disseminated-VX remaining identifiable in the form of VX was 42 h (95% CI, 25–60 h) because that time interval includes the initial rapid reaction of 9% of the disseminated-VX. The reactions that caused fixation of VX occurred rapidly during the first minute post-dissemination, but much more slowly afterward, as evidenced by the data (Table 7) and the nonlinear slow decline depicted in Figure 8. Although the reactions that led to apparent fixation of VX within grass tissue occurred very rapidly during that first minute post-dissemination, the subsequent rate of fixation may have been delimited by cell lysis and necrosis of damaged grass tissue within the area of droplet spread (Figures 5–7), which comports with the extremely low levels of VX outside the area of droplet spread. Even after 1 h post-dissemination, the proportion of VX remaining in the form of VX had not significantly declined ($p > 0.05$), compared to that present at 1 min post-dissemination. The analytically correct 42 h period for half of disseminated-VX to no longer be extractable as VX is biased by the initial rate of VX fixation in grass tissue compared to the subsequent phytophysiologically-driven rate of VX degradation in combination with the slow rate of evaporation of VX. Phytophysiologically-driven factors appear to be primary influences on the strategically important Effective Half-Life (72 h), operationally beginning 1 min post-dissemination. The Effective Half-Life of VX on grass foliage was determined under controlled experimental conditions as the net effect of factors affecting the persistence of VX after 1 min post-dissemination, including subsequent fixation, transformation, and evaporation.

The U.S. Environmental Protection Agency recommends reentry intervals for agricultural workers who use pesticides, including organophosphates, and those reentry intervals are based on the available toxicity data, concentrations of chemicals used, environmental

conditions, crops being treated, and frequency of treatment (Watson et al., 1992). Watson et al. suggested using the Rapid Screening Hazard (RASH) method to determine the reentry interval for individuals who work with VX. The RASH method is based on the determination of a relative potency factor (RPF), derived as the ratio of toxicity data for a reference chemical that has an established reentry interval to toxicity data for a chemical without an established reentry interval. The ratio of the respective RPF values can then be used to calculate a reentry interval. Using the RASH method, the reentry interval post-dissemination for VX would be 60 to 90 days (Haley et al., 2017); however, the RASH method that uses RPF values for pesticide exposures of agricultural workers appears to be unrealistic for extrapolating to the exposure of Warfighters under battlefield conditions.

Historic outdoor studies on Carroll Island (Aberdeen Proving Ground, MD) of the persistence and related toxicity of VX disseminated onto sod (grass) were confounded by changing and uncontrollable environmental conditions (Reich, 1959a, 1959b). However, when the effects of uncontrolled rainfalls (Haley et al., 2016, 2017) and temperature (Tevault et al., 2012) are taken into account, the results presented in this report comport with the general trends that were observed historically. This outcome illustrates the usefulness of the present methods for investigating the fate, persistence, and Effective Half-Life of additional classes of CWAs on plants, providing reliable useful input for models that predict outcomes using established scientific and empirical relationships. The results and data presented herein establish experimental determinations under controlled conditions for the fate and persistence of VX on grass foliage for comparison to model outcomes, and critical information for decision-making affecting Soldiers on VX-contaminated battlefields.

## 5. CONCLUSIONS

The primary focus of this investigation was experimental determination of the Effective Half-Life of VX on grass foliage (*E. crus-galli*) using living healthy plants to obtain results applicable to VX-contaminated battlefields. We evaluated the amount of VX persisting on the foliage of living grass plants as a function of time post-dissemination under controlled measured environmental conditions within a surety hood.

The Effective Half-Life of VX on grass foliage was determined as the net effect of factors affecting the persistence of stabilized CASARM-grade VX on grass, including fixation, transformation, and evaporation. The fate of VX on grass foliage was established as the partitioned percentages of stabilized CASARM-grade VX.

The strategically important Effective Half-Life of VX on grass foliage occurred at a 72 h period post-dissemination (95% CI, 46–98 h), on the basis of comparison to the 88% of VX that was extractable from foliage in the form of VX 1 min post-dissemination. The Effective Half-Life of VX is a key strategic value because it accounts for persistent hazard from the bulk of the disseminated-VX, and corresponds to the slow decline of VX within grass foliage.

Approximately 9% of the disseminated-VX became unavailable upon deposition due to rapid reaction with grass foliage; however, 88% of the disseminated-VX remained in

grass tissues at 1 min post-dissemination; after 1 h post-dissemination the situation was not significantly different. Although half of the VX disseminated onto living grass foliage became unextractable after approximately 42 h (95% CI, 25–60 h), that interval is based on total VX disseminated and includes the 9% of the disseminated-VX that rapidly reacted to result in fixation within grass foliage.

The results reported herein comport with historic outdoor studies of VX disseminated onto sod (grass), when the effects of the outdoor uncontrolled rainfalls and temperature are taken into account.

Experimental data herein were established under controlled measured conditions to ascertain the fate and persistence of VX on grass foliage for input into predictive models and comparison to model outcomes, and provide critical information for decision-making affecting Warfighters on VX-contaminated battlefields.

The newly established methods for investigating agent–plant interactions will be useful for establishing the fate, persistence, and Effective Half-Life of additional classes of CWAs on plants, as well as provide additional useful input for predictive models and strategic battlefield decisions.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ACU | Army Combat Uniform |
| ANOVA | analysis of variance |
| ASTM | American Society for Testing and Materials |
| BDL | below detection limit |
| CAS | Chemical Abstracts Service |
| CASARM | Chemical Agent Standard Analytical Reference Material |
| CI | 95% confidence interval |
| CWA | chemical warfare agent |
| EC | Environment Canada |
| ECBC | U.S. Army Edgewood Chemical Biological Center |
| EMPA | ethyl methyl phosphonic acid |
| EtOAc | ethyl acetate |
| *g* | force of gravity |
| GC | gas chromatography |
| HPLC | high-performance liquid chromatography |
| IPA | isopropyl alcohol |
| LSD | least-significant difference |
| MPA | methylphosphonic acid |
| MRM | multiple reaction monitoring |
| MS | mass spectrometry |
| MS/MS | tandem mass spectrometry |
| *N* | number of replications |
| NMR | nuclear magnetic resonance spectroscopy |
| NRCS | Natural Resources Conservation Service |
| *p* | probability |
| $r^2$ | coefficient of determination |
| RASH | Rapid Screening Hazard |
| regen/extract | fluoride ion regeneration extract |
| RCF | relative centrifugal force |
| RPF | relative potency factor |
| SD | standard deviation |
| SPE | solid-phase extraction |
| *t* | time |
| TOP | Test Operations Procedure |
| VX | *O*-ethyl-*S*-(2-diisopropylaminoethyl) methyl phosphonothiolate |
| VX-$d_5$ | deuterium-labeled VX |
| VX-G | *O*-ethyl methylphosphofluoridate |
| VX-G-$d_5$ | deuterium-labeled VX-G; $^2H_5$-*O*-ethyl methylphosphofluoridate |
| v/v | volume-to-volume ratio |

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## APPENDIX

## COMPARISON OF LOGISTIC GOMPERTZ MODEL AND EXPONENTIAL MODEL OUTCOMES

There were two mathematical models, among those described in Guidance Document EPS 1/RM/46 (EC, 2005), that provided good fits to data for VX extracted from grass leaves as a function of time post-dissemination (Table A1).

Table A1. Parameters for Nonlinear Regression Mathematical Models for Determining the Value of the Effective Half-Life of VX on Grass Foliage, from 1 Min Post-Dissemination Onward

| Parameter | Logistic Gompertz Model | Exponential Model |
|---|---|---|
| Effective Half-Life (h) | 72 | 30 |
| 95% confidence interval | 46–98 | 17–44 |
| Raw $r^2$ | 0.988 | 0.988 |
| Mean corrected $r^2$ | 0.913 | 0.906 |

These models were logistic (Gompertz; eq A1) and exponential (eq A2):

$$Y = a \times e^{([\log(1-p)] \times [H/ETp]b)} \quad \text{(A1)}$$

$$Y = a \times e^{\{([\log(1-p)] / ETp) \times H\} + b} \quad \text{(A2)}$$

In these models,

$Y$ = number for a measurement endpoint (i.e., percent recovery),
$a$ = Y-axis intercept,
e = base of the natural logarithm,
$p$ = desired value for "p" percent recovery (e.g., 0.5 for 50% recovery),
H = time duration (hours) of the recovery,
ETp = estimate of time (hours) for a specified percent recovery, and
$b$ = scale parameter that defines the shape of the equation.

Although the results for both of these models are included in this appendix, the logistic Gompertz model was preferred because it had a greater $r^2$ value for the mean corrected coefficient of determination for the data, and it yielded the greater value of 72 h for the Effective Half-Life as compared with the exponential model outcome (Table A1): Experimental results showed that approximately 57% of VX extractable at 1 min post-dissemination remains extractable at 48h, yet the exponential model predicted that by 30 h that half of the VX that was extractable at 1 min would no longer be extractable. Compared to the exponential model estimate, the value for the Effective Half-Life of VX on grass foliage from 1 min post-dissemination onward as estimated by the Gompertz logistic model provides a more accurate (compared to 48 h mid-time-point data), conservative, safer estimate for the risk of exposure on the battlefield that may be associated with a persistent hazard from the bulk of disseminated-VX.

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